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A hut of the stone age discovered at Schussenried (Wurtemburg). By Dr. V. Gross. *Matériaux*, XVII, 321–323.

The horse in prehistoric and historic times. By C. A. Pietrement. *Matériaux*, XVII, 323–336.

MICROSCOPY AND HISTOLOGY.[1]

PURE CARMINIC ACID FOR COLORING MICROSCOPICAL PREPARATIONS.[2]—Since Gerlach first called attention to carmin as a coloring matter for histological purposes, many methods of preparing the carmin solutions have been published, but, so far as I am aware, none which result in anything but mixtures of various carmin salts with undetermined and undesirable impurities, such as fatty matter, tyrosin, sugar, and salts of alkaline metals. Of course the coloration produced by these mixtures has been sometimes good, sometimes bad, and the solutions have easily spoiled or decomposed, while their preparation often required considerable time and trouble. Several years ago I studied carmin, chiefly from a chemical standpoint, and since then I have often wondered why naturalists usually used carmin solutions in which water, with some caustic or destructive material added, was the principal solvent. Carmin of commerce, it is true, is not readily soluble, even in water, until ammonia, borax, or some other aid to solution is added; but carminic acid, the basis of the coloring matter of carmin, has long been stated in the leading chemical dictionaries and hand-books to be readily soluble in water and in alcohol. Watts (Dict. Chem., 1872, 1st suppl., p. 413) says of carminic acid: "This acid forms a purple mass, fusible and soluble in all proportions in water and in alcohol. Sulphuric and hydrochloric acid dissolve it without alteration. It bears a heat of 136° C. without decomposition."

Earlier still Watts (Dict. Chem., 1863, v. 1, p. 804) says: "The fine red pigment known in commerce as carmin is prepared by treating a solution of cochineal with cream of tartar, alum, or acid oxalate of potassium. The fatty and albuminous matters then coagulate and carry down the coloring matter with them." Now in preparing most carmin solutions this precipitation takes place, and the carmin, having greater cohesive (not chemical) affinity for impurities of animal origin than for alcohol, its solution is not readily accomplished by that medium, nor indeed by water. In preparing carmin solutions for histological purposes by some of the published recipes, more than one-half of the coloring matter of the carmin is lost in the refuse left upon the filter paper.

There are two ways commonly in use for preparing carminic acid, which term I here use as distinguished from "acid carmin" (the cochineal decoction for which Grenacher, Schweigger-Seidel, and others have given recipes) and from carmin (the impure commercial powder). The first mode of preparation, the one which I followed in preparing the acid upon which I have experimented is that of Warren De la Rue. Watts, whom I quote because he gives clear directions (Dict. Chem., 1863, vol. 1, p. 804), gives De la Rue's method, as follows: "To separate it [carminic acid] cochineal is exhausted with boiling water; the extract is precipitated by subacetate of lead slightly acidulated, care being taken not to add the lead-solution in excess; the precipitate is washed with distilled water till the wash-water no longer gives a precipitate with a solution of mercuric chloride, then decomposed by sulphuretted hydrogen; the filtrate is evaporated to a syrupy consistence and dried over the water-bath; and the dark purple product thus obtained is treated with alcohol which extracts the carminic acid." The second mode of preparing carminic acid, that of Schaller, is given by Watts (Dict. Chem., 1872, 1st suppl., p. 413) as follows: "C. Schaller prepares this acid by precipitating the aqueous extract of cochineal with neutral lead acetate slightly acidulated with acetic acid; decomposing the washed precipitate with sulphuric acid; again precipitating the filtrate with lead-acetate, and decomposing the precipitate with sulphuric acid, avoiding an excess; then precipitating a third time, and decomposing the precipitate with hydrogen sulphide. The filtered solution is evaporated to dryness; the residue dissolved in absolute alcohol; the crystalline nodules of carminic acid obtained on leaving this solution to evaporate are freed from a yellow substance by washing with cold water, which dissolves only the carminic acid; and the residue left on evaporating the aqueous solution is recrystallized from absolute alcohol or from ether."

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[1] Edited by Dr. C. O. WHITMAN, Mus. Comparative Zoology, Cambridge, Mass.

[2] By Geo. Dimmock. Read at the New York meeting of the Society of Naturalists of the Eastern United States, 28th Dec., 1883.

Schaller's mode of preparation gives purer carminic acid than De la Rue's, but either kind is sufficiently pure for histological purposes. The precipitation by lead acetate and the dissolving in alcohol free the carminic acid from animal impurities, and the consequence is a purer form of pigment than can be extracted by any process hitherto employed for the preparation of carmin for histological purposes. I will here add that carminic acid has been shown recently by Liebermann and Van Dorp to be related, through nitrococcusic acid, to trinitrocresol, which has been obtained from coal-tar cresol, thus showing that the coloring matter of cochineal contains methylated benzine-residues.

I do not know whether carminic acid, ready prepared, is obtainable. Any chemical student could easily prepare it by the processes given above.

It is unnecessary to explain to naturalists the advantages of alcoholic solutions of carmin over aqueous ones. The alcoholic solution colors preparations much quicker than the aqueous solution does; for coloring sections I employ a solution of 0.25 gram carminic acid to 100 grams of eighty per cent alcohol, and leave sections in the solution from two to five minutes. A solution of equal carmin strength but in absolute alcohol can be employed; it has, however, no special advantages, since with the eighty per cent alcoholic solution the sections can be washed directly in absolute alcohol, and then put into oil of cloves or turpentin. Coloring in the piece before sectioning never takes as long with alcoholic carminic acid as it does with ordinary carmin solutions, and if it did take long the strong alcohol would preserve the tissue from maceration. In coloring pieces of mollusca, or of other equally slimy animals, the slime should be removed beforehand, or the coloration will be unsatisfactory, because the slime congealing in the alcohol takes up the coloring matter, forming an almost impervious colored layer on the outside and leaving the inside of the piece nearly uncolored.

Some preparations colored in alcoholic carminic acid and then put up in glycerin lost their color in a few months, the color seeming to be entirely diffused in the glycerin, while similar preparations mounted in Canada balsam retained their color perfectly. I do not know if this fading would occur with preparations colored with alcoholic ammonic carminate, or even if this diffusion was not due to some impurity of the glycerin (of the purity of which I was doubtful); time to test this matter further failed.

An alcoholic ammonic carminate, or ammonia carmin, can be prepared, at a moment's notice, from alcoholic carminic acid, by adding ammonia, drop by drop, and stirring until the entire solution changes from its bright red to purple red. By this mode pure alcoholic ammonic carminate can be produced with no excess of ammonia, and at any time. As the carminic acid can be preserved dry without decomposition, and dissolves quickly in alcohol, one can carry the ingredients of a carmine solution in the vest pocket, without inconvenience.

In making and using alcoholic carminic acid pure alcohol and distilled water give the best results, because a portion of the carminic acid is converted to carminates by the salts of impure water. In making alcoholic ammonic carminate this precaution is not as necessary, because the color of the carminates produced by the impurities of the water is so nearly like that of ammonic carminate.

Alcoholic carminic acid may be used, as Grenacher's carmin solution is used, to color sections from which the coloration is to be afterwards partly extracted by very dilute hydrochloric acid, leaving nuclei red. Another way to use carmin solutions, which is especially applicable to alcoholic carminic acid, is to precipitate the carmin in the tissues by some salt, the carminate of the base of which gives a desired coloration. I have found, for example, that specimens hardened for a moment under the cover-glass with an alcoholic solution of corrosive sublimate (mercuric chloride) and, after washing with alcohol, colored in alcoholic carminic acid, took a fine coloration of mercuric carminate. So, too, specimens colored in alcoholic carminic acid can be changed by a few moments' treatment with a *very dilute* alcoholic solution of lead-acetate or cobalt nitrate to a beautiful purple. With lead acetate used as above a double coloration is sometimes produced, but I have not examined sufficiently these colorations to accurately describe them. Cupric and other salts, used as above described, have not given me very favorable results. Sometimes salts in the tissues of the animals themselves change portions of the carminic acid to purple carminates, giving a double coloration without further treatment.

Picric acid added to alcoholic carminic acid *in extremely small quantities* (best in a dilute alcoholic solution, testing the solution on specimens after each addition) makes a double alcoholic coloring fluid (a so-called picro-carmin). I have been unable thus far to determine the proportion of picric acid required for this solution, having in every case added an excess. All different kinds of carmin solutions can be made from carminic acid with the advantage of having always uniform strength, of being definite mixtures, and of not spoiling as readily as those made directly from cochineal.

Incompatible reagents with carminic acid are, of course, all alkaline solutions and nearly all metallic salts; with ammonic carminate, are naturally all acids; with all carmine solutions, are bromine and chlorine.

I hope later to try some coloring experiments with coccinin, ruficoccin, carmin red, and ruficarmin, all of which are derived from carminic acid, and of possible value to the histologist.

STRUCTURE OF SECRETORY CELLS AND THEIR CHANGES IN SECRETION.—The histological structures of the various secretory cells have been described by different observers, as well as the changes undergone in them during the act of secretion. But these descriptions exhibit many discrepancies, which are largely due to the differences between the methods employed in studying the tissues. Mr. J. N. Langley, whose experience eminently qualifies him for such a task, undertakes to combine all that is true in our conceptions of the secretory cell. He says: "The glands of vertebrates in which I find that the secretory cells have fundamentally the same structure are—the serous and mucous salivary glands, and the similar glands of the mucous membrane of the mouth, nose, larynx, œsophagus, etc.; with a few exceptions, the 'chief' cells of mammalian gastric glands; the gastric glands of such birds, fishes, reptiles and amphibia as I have examined; the œsophageal glands of the frog; the pancreas; the liver. * * * * The secretory cells of all the glands in the list which I have given have the following common points of structure: The cell substance is composed of *(a)* a framework of living substance or protoplasm, connected at the periphery with a thin continuous layer of modified protoplasm; the framework in some cases has the form of a network of small threads of equal size as described by Klein, in others of flattened bands. Further, the threads or bands may vary in size in different parts of the cell, and the meshes in different parts of the cell may be of different size and shape. Within the meshes of the framework are enclosed two chemical substances at least, viz., *(b)* a hyaline substance in contact with the framework, and of *(c)* spherical granules which are embedded in the hyaline substance. In the gland cells which secrete much organic matter the cell granules are conspicuous and fairly large. In the gland cells which secrete comparatively little organic matter the cell-granules are, generally speaking, smaller and less distinct, the lower the mean percentage of organic matter is in the fluid secreted. The cell-granules are in nearly all cases mesostales, *i. e.*, substances stored up in the cell and destined to give rise to the organic substances of the secretion. The granularity of a cell in the resting state thus depends upon its storage power. Generally speaking, the greater the storage power of a cell, the higher is the percentage of the organic substance in its secretions, but this is not always the case, since it may happen that the rate of secretion of water may increase without any corresponding increase in the rate of secretion of organic substance, and in consequence the percentage of organic substance in the secretion may be small; further, it is possible that under special circumstances a cell with small storage power might secrete a large quantity of its stored-up material, and that a cell with a large storage power might secrete a very small quantity of its stored-up material, the amount of water secreted by the two cells being approximately equal. In all these cells, during active secretion, the following changes take place: The granules decrease in number, and usually, if not always, in size; the hyaline substance increases in amount; the network grows. The increase of the network is much less than that of the hyaline substance. Moreover, in the majority of cells the details of the changes which take place are much the same. The hyaline substance increases chiefly in the outer region of the cells, and the granules disappear from this region, so that an outer non-granular zone and an inner granular zone are formed. The network stretches throughout the cell in all cases. In the outer zone its meshes are filled with hyaline substance; in the inner zone its meshes are filled with granules and a small amount of hyaline substance. The glands in which an outer non-granular zone is not formed during secretion are most of the gastric glands of the frog and toad, the gastric glands of the snake, and the liver of mammals. * * * * It is to be remembered that there is reason to believe that the three parts of the cell are continually being formed and changed into other substances. * * * The differences shown by the different cells after the same treatment depends partly upon the different chemical characters of the framework, hyaline substance, and granules in the different cells, and partly upon the different arrangement of these constituents. * * * An obvious hypothesis is that the protoplasmic network forms the hyaline substance, and then out of this manufactures the granules, which are, as we know, converted during secretion into some one or more of the organic bodies of the fluid secreted. * * * The network appears to be the result of the two-fold tendency of the protoplasm to form fibrillæ, and to store up substances within its grasp; in most cases it is obviously not constant in form, but is continually altering the arrangement of its bars and the size of its meshes. This is especially distinct in mucous cells in which, during secretion, numerous fresh connecting fibrillæ are formed."—*Proc. Camb. Philos. Soc., Vol. v.*

PROCEEDINGS OF THE AMERICAN SOCIETY OF MICROSCOPISTS.—This volume of 275 pages contains the proceedings of the sixth meeting of this active society, which was held at Chicago, Aug. 7–10, 1883. It has been edited by Dr. D. S. Kellicott, and has certainly been issued with promptness. The address of President McCalla was on the verification of microscopic observation. The specially microscopical articles were the following: College microscopical societies, by Sarah F. Whiting; the relation of aperture to amplification in the selection of a series of objectives, by Geo. E. Blackham; division of labor among microscopists, by J. M. Mansfield; photo-micrography with dry plates and lamp light, by W. H. Walmsley; the preparation and mounting of Foraminifera, with description of a new slide for opaque objects, by F. M. Hamlin; preparing and mounting bacteria, by T. J. Burrill; the microscope in the detection of lard adulterations, by W. T. Belfield; a new method of dry mounting, by A. H. Chester; a new form of microscope stand with concentric movements, by J. D. Cox; a critical study of the action of a diamond in ruling lines upon glass, by W. A. Rogers; cataloguing, labeling, and storing microscopical preparations, by S. H. Gage. There are also reports of the committee on eye-pieces, micrometry, centimeter scale A, 1882, a study of centimeter, marked A, prepared by the U. S. Bureau of Weights and Measures, by W. A. Rogers, and rules for the control of the standard micrometer.

"THE MICROSCOPICAL BULLETIN."—The first number of the new *Microscopical Bulletin*, to be published bi-monthly by James W. Queen & Co., Philadelphia, appeared in December. This publication is designed to answer the question, "What's new in the microscopical line?" The first number consists of eight double-column octavo pages, and contains the more interesting portions of Professor Duncan's address before the Roy. Mic. Soc., in 1882; a short essay on pathogenic bacteria, a description of a new slide-box, and other microscopic accessories.